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(54) Title: METHODS FOR MONITORING EFFECTIVENESS OF INTERFERON THERAPY IN INDIVIDUALS WITH HCV INFECTIONS

(57) Abstract

A means for determining the effectiveness of interferon therapy in individuals who are infected with Hepatitis C Virus (HCV) and are receiving such therapy. Monitoring the level of anti-HCV IgM and/or anti-GOR IgG provide means for establishing whether or not the infected patient is responding to interferon therapy; such monitoring is especially useful for patients diagnosed with chronic acute HCV infections.

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# METHODS FOR MONITORING EFFECTIVENESS OF INTERFERON THERAPY IN INDIVIDUALS WITH HCV INFECTIONS

## Background of the Invention

This invention relates generally to interferon therapy, and more particularly, relates to the monitoring of anti-HCV IgM or anti-GOR IgG in an individual's test sample as an indication of the clinical effectiveness and/or clinical outcome of interferon therapy used to treat an individual with chronic HCV infection.

Greater than 90% of cases of transfusion hepatitis worldwide are attributed to non-A, non-B hepatitis (NANBH). The predominant etiological agent of NANBH, termed Hepatitis C virus (HCV), has been cloned. An immunodominant region designated as c-100, encoded by the putative nonstructural (NS)-4 genomic region, has been expressed, purified, and incorporated into immunoassays which are useful in the detection of antibody to HCV in infected test samples. See, for example, Q.-L. Choo et al., Science 244:359-362 (1989); H. J. Alter et al., N. Engl. J. Med. 321:1494-1500 (1989); J. I. Esteban et al., Lancet ii:294-297 (1989); G. Huo et al., Science 244:362-364 (1989); T. Miyamura et al., Proc. Natl. Acad. Sci. USA 87:983-987 (1990); and C. L. Van der Poel et al., Lancet ii:297-298 (1989).

Recently, Mishiro et al. have reported isolation of a cDNA clone designated as GOR4701, from the plasma of a chimpanzee experimentally infeted with NANBH agent. S. Mishiro et al., Lancet 336:1400-1403 (1990). the GOR-47-1 cDNA clone was shown to lack detectable sequence homology to known HCV sequences. Further, unlike HCV, GOR gene is coded by a single-copy gene of host cellular sequence. An EIA has been developed using a 27-amino acid synthetic peptide, spGOR2, deduced from the cDNA sequence of the GOR gene. Using this EIA. 60% to 80% if the samples were found to be reactive, while only two percent (2%) of the voluntary donor population had anti-GOR2 antibodies, suggesting a strong association between immune response to GOR and HCV in NANBH patients.

It is known that a percentage of individuals who develop NANBH (HCV) infections progress to the chronic state of the disease, where liver function is impaired and death ultimately may result in severe cases. Interferon therapy has been shown to improve liver function in a proportion of patients with NANB hepatitis. About fifty percent (50%) of chronic Hepatitis C cases respond to alpha interferon (IFN) therapy. No demographic, biochemical or clinical features have been identified to predict response to therapy. Interferon induced normalization of serum ALT levels is so far the only endpoint indicating response to IFN therapy. Biochemical relapse is predicted by increasing serum ALT levels. In addition to ALT elevation, recurrence of HCV viremia as measured by PCR during or after interferon treatment is discussed as a possible predictor for relapse. However, ALT elevations may not occur for several months after viremia recurrence. It is not clear at this time if changes in anti-HCV IgG levels are associaterd with IFN treatment.

It would be advantageous to provide a means for predicting the clinical outcome of interferon therapy in an individual receiving such therapy for NANB (HCV) infection. Such a means would be useful in that the response to therapy could be monitored, and the state of disease could be assessed to determine remission or relapse after therapy.

Summary of the Invention

The present invention provides a means for determining the effectiveness and/or possible clinical outcome of interferon therapy when it is used in individuals diagnosed with chronicHCV infection, or when interferon therapy is used as a treatment for an HCV infection. Briefly, test samples from an individual are taken before the start of interferon therapy and during the course of interferon therapy, and assayed for either anti-HCV IgM and/or anti-GOR IgG antibodies. A decrease in either anti-HCV IgM level or anti-COR IgG level in indicative of a clinical response to interferon therapy.

Several assay procedures disclosed herein can be used to assay for anti-HCV IgM activity. HCV antigens useful in these assays include HCV CORE, HCVp33c and HCV c-100. The most preferred antigen is HCV CORE. These assays can include a solid phase to

which HCV antigen is attached. The solid phase selected can include polymeric or glass beads, nitrocellulose, microparticles, wells of a reaction tray, test tubes and magnetic beads. The signal generating compound can include an enzyme, a luminescent compound, a chromogen, a radioactive element and a chemiluminescent compound. Examples of enzymes include alkaline phosphatase, horseradish peroxidase and beta-galactosidase. Examples of enhancer compounds include biotin, anti-biotin and avidin. Examples of enhancer compound binding members include biotin, anti-biotin and avidin. In order to block the effects of rheumatoid factor-like substances, the test sample is subjected to conditions sufficient to block the effect of rheumatoid factor-like substances. These conditions comprise contacting the test sample with a quantity of anti-human IgG to form a mixture, and incubating the mixture for a time and under conditions sufficient to form a reaction mixture product substantially free of rheumatoid factor-like substance.

Several assay procedures disclosed herein also can be used to assay for anti-GOR IgG activity. The GOR antigen most preferred in this assay is GOR2. These assays can include a solid phase to which GOR antigen is attached. The solid phase selected can include polymeric or glass beads, nitrocellulose, microparticles, wells of a reaction tray, test tubes and magnetic beads. The signal generating compound can include an enzyme, a luminescent compound, a chromogen, a radioactive element and a chemiluminescent compound. Examples of enzymes include alkaline phosphatase, horseradish peroxidase and beta-galactosidase. Examples of enhancer compounds include biotin, anti-biotin and avidin. Examples of enhancer compound binding members include biotin, anti-biotin and avidin. In order to block the effects of rheumatoid factor-like substances, the test sample is subjected to conditions sufficient to block the effect of rheumatoid factor-like substances. These conditions comprise contacting the test sample with a quantity of anti-human IgG to form a mixture, and incubating the mixture for a time and under conditions sufficient to form a reaction mixture product substantially free of rheumatoid factor-like substance.

Detailed Description of the Invention

The present invention provides a means for determining the effectiveness of interferon therapy for individuals receiving such therapy for HCV infections. The inventions comprises assaying for the presence of anti-GOR IgG and/or anti-HCV IgM and using these determinations to determine clinical effectiveness and/or clinical outcome of an individual.

The present invention employs an immunoassay which utilizes specific binding members. A "specific binding member," as used herein, is a member of a specific binding pair. That is, two different molecules where one of the molecules through chemical or physical means specifically binds to the second molecule. Therefore, in addition to antigen and antibody specific binding pairs of common immunoassays, other specific binding pairs can include biotin and avidin, carbohydrates and lectins, complementary nucleotide sequences, effector and receptor molecules, cofactors and enzymes, enzyme inhibitors and enzymes, and the like. Furthermore, specific binding pairs can include members that are analogs of the original specific binding members, for example, an analyte-analog. Immunoreactive specific binding members include antigens, antigen fragments, antibodies and antibody fragments, both monoclonal and polyclonal, and complexes thereof, including those formed by recombinant DNA molecules. The term "hapten", as used herein, refers to a partial antigen or non-protein binding member which is capable of binding to an antibody, but which is not capable of eliciting antibody formation unless coupled to a carrier protein.

A "capture reagent", as used herein, refers to an unlabeled specific binding member which is specific either for the analyte as in a sandwich assay, for the indicator reagent or analyte as in a competitive assay, or for an ancillary specific binding member, which itself is specific for the analyte, as in an indirect assay. The capture reagent can be directly or indirectly bound to a solid phase material before the performance of the assay or during the performance of the assay, thereby enabling the separation of immobilized complexes from the test sample.

Test samples which can be tested by the methods of the present invention described herein include human and animal body fluids such as whole blood, serum, plasma, cerebrospinal fluid, urine, biological fluids such as cell culture supernatants, tissue specimens and cell specimens.

An enhancer can be used to detect the generated signal in the assay. By "enhancer" is meant a moiety which can bolster a signal generated in an immunoassay, thereby amplifying the generated signal. Several methods of enhancing and amplifying a signal generated in an immunoassay are known in the art. Also, the use of a signal enhancer such as the use of avidin-biotin also is known. For example, U. S. Patent No. 4,228,237 to Hevey et al. describes the use of a biotin labelled specific binding substance for a ligand used in a method which also employs an enzyme labelled with avidin. The use of a biotin-anti-biotin system is described in European Patent Application No. 160,900, published on November 13, 1985.

The term "probe," as used herein, means a member of the specific binding pair attached to an "enhancer" compound. An "enhancer" compound can be any compound used in the assay which can enhance the signal generated by the signal generating compound. Thus, enhancer compounds include haptens such as biotin, and also include fluorescein, di-nitrophenol, and the like.

The indicator reagent comprises a signal generating compound (label) which is capable of generating a measurable signal detectable by external means conjugated (attached) to a specific binding member. "Specific binding member," as used herein, means a member of a specific binding pair. That is, two different molecules where one of the molecules through chemical or physical means specifically binds to the second molecule. In addition to being an antibody member of a specific binding pair for HCV, the indicator reagent also can be a member of any specific binding pair, including either hapten-anti-hapten systems such as biotin or anti-biotin, avidin or biotin, a carbohydrate or a lectin, a complementary nucleotide sequence, an effector or a receptor

molecule, an enzyme cofactor and an enzyme, an enzyme inhibitor or an enzyme, and the like. An immunoreactive specific binding member can be an antibody, an antigen, or an antibody/antigen complex that is capable of binding either to HCV as in a sandwich assay, to the capture reagent as in a competitive assay, or to the ancillary specific binding member as in an indirect assay. Thus, if an enhancer is utilized in the assay, the indicator reagent comprises a signal generating compound conjugated to an enhancer-specific compound (enhancer compound binding member), such as biotin or anti-biotin, avidin or biotin, and others known to those skilled in the art. For example, if the enhancer compound utilized is biotin, then anti-biotin, or avidin, can be used as the enhancer-specific compound.

The various signal generating compounds (labels) contemplated include a chromogen such as bromo-chloro-indole-phosphate (BCIP), catalysts such as enzymes, luminescent compounds such as fluorescein and rhodamine, chemiluminescent compounds such as acridinium, phenanthridinium or 1,2-dioxetane compounds, radioactive elements, and direct visual labels. Examples of enzymes include alkaline phosphatase, horseradish peroxidase, beta-galactosidase, and the like. The selection of a particular label is not critical, but it will be capable of producing a signal either by itself or in conjunction with one or more additional substances, such as the use of enzyme substrates when enzymes are employed as the signal generating compound.

It is contemplated that the reagent employed for the assay can be provided in the form of a kit with one or more containers such as vials or bottles, with each container containing a separate reagent such as a monoclonal antibody, or a cocktail of monoclonal antibodies, employed in the assay.

The assay configuration may involve the use of a solid phase in performance of the present invention. A "solid phase", as used herein, refers to any material which is insoluble, or can be made insoluble by a subsequent reaction. The solid phase can be chosen for its intrinsic ability to attract and immobilize the capture reagent. Alternatively, the

solid phase can retain an additional receptor which has the ability to attract and immobilize the capture reagent. The additional receptor can include a charged substance that is oppositely charged with respect to the capture reagent itself or to a charged substance conjugated to the capture reagent. As yet another alternative, the receptor molecule can be any specific binding member which is immobilized upon the solid phase and which has the ability to immobilize the capture reagent through a specific binding reaction. The receptor molecule enables the indirect binding of the capture reagent to a solid phase material before the performance of the assay or during the performance of the assay. If an assay device is utilized to perform the assays of the present invention, it can have many configurations, several of which are dependent upon the material chosen as the solid phase. For example, the solid phase can include any suitable porous material. By "porous" is meant that the material is one through which the test sample can easily pass and includes both bibulous and non-bibulous solid phase materials. In the present invention, the solid phase can include a fiberglass, cellulose, or nylon pad for use in a pour and flow-through assay device having one or more layers containing one or more of the assay reagents; a dipstick for a dip and read assay; a test strip for wicking (e.g., paper) or thin layer chromatographic or capillary action (e.g., nitrocellulose) techniques; or other porous or open pore materials well known to those skilled in the art (e.g., polyethylene sheet material). The solid phase, however, is not limited to porous materials. The solid phase can also comprise polymeric or glass beads, microparticles, tubes, sheets, plates, slides, wells, tapes, test tubes, or the like, or any other material which has an intrinsic charge or which can retain a charged substance.

Natural, synthetic, or naturally occurring materials that are synthetically modified, can be used as a solid phase including polysaccharides, e.g., cellulose materials such as paper and cellulose derivatives such as cellulose acetate and nitrocellulose; silica; inorganic materials such as deactivated alumina, diatomaceous earth, $MgSO_4$, or other inorganic finely divided material uniformly dispersed in a porous polymer matrix, with polymers such as vinyl chloride, vinyl chloride-propylene copolymer, and vinyl chloride-vinyl acetate

copolymer; cloth, both naturally occurring (e.g., cotton) and synthetic (e.g., nylon); porous gels such as silica gel, agarose, dextran, and gelatin; polymeric films such as polyacrilamide; and the like. The solid phase should have reasonable strength or strength can be provided by means of a support, and it should not interfere with the production of a detectable signal.

Preferred solid phase materials for flow-through assay devices include filter paper such as a porous fiberglass material or other fiber matrix materials. The thickness of such material is not critical, and will be a matter of choice, largely based upon the properties of the sample or analyte being assayed, such as the fluidity of the test sample.

To change or enhance the intrinsic charge of the solid phase, a charged substance can be coated directly to the material or onto microparticles which are then retained by a solid phase support material. Alternatively, microparticles can serve as the solid phase, by being retained in a column or being suspended in the mixture of soluble reagents and test sample, or the particles themselves can be retained and immobilized by a solid phase support material. By "retained and immobilized" is meant that the particles on or in the support material are not capable of substantial movement to positions elsewhere within the support material. The particles can be selected by one skilled in the art from any suitable type of particulate material and include those composed of polystyrene, polymethylacrylate, polypropylene, latex, polytetrafluoroethylene, polyacrylonitrile, polycarbonate, or similar materials. The size of the particles is not critical, although it is preferred that the average diameter of the particles be smaller than the average pore size of the support material being used.

Solid supports are known to those in the art and include the walls of wells of a reaction tray, test tubes, polystyrene beads, magnetic beads, nitrocellulose strips, membranes, microparticles such as latex particles, chips of glass, plastic, derivatized plastic, metal and silicon, and others.

Accordingly, a test sample which may contain HCV IgM is contacted with a solid support to which HCV antigen has been attached, to form a mixture. This mixture is incubated for a time and under conditions sufficient to form HCV antigen/antibody complexes. Then, a probe comprising a mammalian anti-human IgM to which an enhancer has been attached is contacted with the HCV antigen/antibody complexes, to form a second mixture. This second mixture is incubated for a time and under conditions sufficient to form a second mixture reaction product. Next, an indicator reagent which comprises an enhancer compound binding member and a signal generating compound capable of generating a measurable signal is contacted with the second mixture reaction product . This third mixture is incubated for a time and under conditions sufficient to form indicator reagent reaction products. The presence and/or amount of HCV IgM is determined by detecting the signal generated. The amount of HCV IgM present in the test sample is proportional to the signal generated.

Another assay comprises an assay wherein a test sample which may contain HCV IgM is contacted with a solid support to which HCV antigen has been attached, to form a mixture. This mixture is incubated for a time and under conditions sufficient to form HCV antigen/antibody complexes. Then, an indicator reagent which comprises a signal generating compound capable of generating a measurable signal attached to a specific binding member for HCV IgM is contacted with the complexes, to form a second mixture. This second mixture is incubated for a time and under conditions sufficient to form a reaction. The presence and/or amount of HCV IgM present in the test sample is determined by detecting the signal generated. The amount of HCV IgM present in the test sample is proportional to the signal generated.

In yet another assay configuration, a test sample is contacted with mammalian anti-human IgM which is coated on a solid phase, and reacted for a time and under conditions sufficient for human IgM/anti-human IgM complexes to form. These complexes then are contacted with a probe which comprises at least one HCV antigen

selected from HCV CORE, HCV 33c and HCV c-100 attached to an enhancer compound. The preferred enhancer compound is biotin. These are reacted for a time and under conditions sufficient to form antigen/antibody/antibody complexes. Next, these complexes are contacted with an indicator reagent comprising a signal generating compound conjugated to an enhancer compound binding member. The most preferred signal generating compound is the enzyme alkaline phosphatase. The most preferred enhancer compound binding member is anti-biotin. The resultant mixture is reacted for a time and under conditions sufficient for a reaction to occur. If an enzyme is utilized, the signal is detected and measured after addition of an enzyme substrate. The amount of HCV IgM present in the test sample is proportional to the signal generated.

Yet other assay configurations can be adapted to detect HCV IgM by practicing the teachings of this invention, and are contemplated to be within the scope of this invention.

The presence of anti-GOR IgG antibody also can be assayed and monitored during and after interferon therapy for HCV infection by using the assays disclosed hereinabove and substituting the appropriate GOR antigens. An antibody test for GOR peptides utilizing the synthetic spGOR2 and spGOR346 peptides is known in the art and disclosed in S. U. Mehta et al., J. Clin. Immunology 12(3):178-184 (1992). Thus, a test sample which may contain GOR IgG is contacted with a solid support to which GOR antigen has been attached, to form a mixture. This mixture is incubated for a time and under conditions sufficient to form GOR antigen/antibody complexes. Then, a probe comprising a mammalian anti-human IgG to which an enhancer has been attached is contacted with the GOR antigen/antibody complexes, to form a second mixture . This second mixture is incubated for a time and under conditions sufficient to form a second mixture reaction product. Next, an indicator reagent which comprises an enhancer compound binding member and a signal generating compound capable of generating a measurable signal is contacted with the second mixture reaction product . This third mixture is incubated for a time and under conditions sufficient to form indicator reagent reaction products. The

presence and/or amount of GOR IgG is determined by detecting the signal generated. The amount of GOR IgG present in the test sample is proportional to the signal generated.

Another assay comprises an assay wherein a test sample which may contain GOR IgG is contacted with a solid support to which GOR antigen has been attached, to form a mixture. This mixture is incubated for a time and under conditions sufficient to form GOR antigen/antibody complexes. Then, an indicator reagent which comprises a signal generating compound capable of generating a measurable signal attached to a specific binding member for GOR IgG is contacted with the complexes, to form a second mixture. This second mixture is incubated for a time and under conditions sufficient to form a reaction. The presence and/or amount of GOR IgG present in the test sample is determined by detecting the signal generated. The amount of GOR IgG present in the test sample is proportional to the signal generated.

In yet another assay configuration, a test sample is contacted with mammalian anti-human IgG which is coated on a solid phase, and reacted for a time and under conditions sufficient for human IgG/anti-human IgG complexes to form. These complexes then are contacted with a probe which comprises at least one GOR antigen selected from spGOR346 and spGOR2 attached to an enhancer compound. The preferred enhancer compound is biotin. These are reacted for a time and under conditions sufficient to form antigen/antibody/antibody complexes. Next, these complexes are contacted with an indicator reagent comprising a signal generating compound conjugated to an enhancer compound binding member. The most preferred signal generating compound is the enzyme alkaline phosphatase. The most preferred enhancer compound binding member is anti-biotin. The resultant mixture is reacted for a time and under conditions sufficient for a reaction to occur. If an enzyme is utilized, the signal is detected and measured after addition of an enzyme substrate. The amount of GOR IgG present in the test sample is proportional to the signal generated.

Yet other assay configurations can be adapted to detect GOR IgG by practicing the teachings of this invention, and are contemplated to be within the scope of this invention.

When assaying for HCV anti-IgM, it is preferred that the test sample be treated such as to remove rheumatoid factor-like substances which may be present in the test sample and which may interfere with the performance of the assay. Such treatment can be performed in a variety of ways known to those skilled in the art and include preadsorbing the test sample with protein A or protein G, heat aggregated IgG, and the subjection of the test sample to an amount of anti-human IgG sufficient to bind a substantial amount of the interfering rheumatoid factor-like substances. The most preferred method for treating the test sample comprises diluting the test sample in a diluent sample buffer which contains an amount of goat anti-human IgG sufficient to bind the rheumatoid factor-like substances which may be present in the test sample. This dilution step preferably is performed prior to contacting the test sample with the capture reagent, HCV antigen. The preferred buffer is one which can remove any interfering IgG which may be present in the test sample. Thus, buffers which contain a sufficient quantity of anti-IgG can be used as the diluent sample buffer. Examples of buffers that can be used in the assay include Tris buffered saline, phosphate buffered saline, and others known to those skilled in the art. The most preferred buffer comprises a Tris buffered saline (pH 7.2) to which goat anti-human IgG has been added. Further, other compounds may be added to this buffer to block non-specific binding. The selection of these compounds depends upon the constituents chosen for the assay, and are within the ordinary skill of the artisan.

The origin of the mammalian anti-human IgM or anti-IgG may be goat, rabbit, sheep, or other mammalian anti-human IgM known in the art. Preferably, the mammalian origin of the anti-human IgM and anti-human IgG is goat.

When HCV or GOR antigen is used as a capture reagent in the assays described herein, at least one HCV or GOR antigen is used,

either when attached to a solid phase or in solution. These HCV antigens include HCV CORE, HCV 33c and HCV c-100. The GOR antigens include GOR2 and GOR 346. We have determined that HCV CORE is the most preferred antigen to utilize in performing the assay for detection of anti-HCV IgM antibodies , but that HCV 33c and HCV c-100 also can be used alone or in any combination. Thus, HCV CORE antigen can be combined with HCV 33c and/or c-100, or other HCV antigens, and used as capture antigens in methods described herein.

It also is contemplated that a sandwich assay can be performed wherein a soluble capture reagent can include an analyte-specific binding member which has been bound to a charged substance such as an anionic substance. The present invention also can be used to conduct a competitive assay. In a competitive configuration, the soluble capture reagent again includes a specific binding member which has been attached to a charged substance, such as an anionic polymer, with which to bind a specific binding partner.

Alternatively, it also is contemplated that the assay can be performed by scanning probe microscopy, in which an analyte, analyte analog or analyte specific substance which has been bound to a test piece, is contacted with the test sample suspected of containing the analyte, incubated for a time and under conditions sufficient for a reaction to occur, and then the presence of analyte is determined by using scanning probe microscopy.

The present invention will now be described by way of Examples, which are intended to demonstrate, but not to limit, the spirit and scope of the invention.

## EXAMPLES

### Example 1

### Anti-IgM Activity

1. Patient Selection and Categorization

Thirty-four (34) patients with chronic active hepatitis C at different time points during interferon therapy (1 to 5 million units of interferon for at least six (6) months) were included. Complete responders (CR) (11/34) were defined by normalization of serum

transaminases during treatment. Non-responders (NR) (23/34) were defined as those individuals who showed transaminases levels above two (2) times the normal values (28 IU/ML). In total, 119 samples were tested for HCV IgM antibodies. Samples were collected approximately one (1) month before, two (2) months after the beginning of and at the completion of IFN therapy. In a number of patients, samples drawn approximately six (6) months after therapy also were available.

2. HCV Detection in Samples

All samples initially were tested by the Abbott HCV 2nd Generation assay EIA and the Abbott HCV Supplemental EIA (available from Abbott Laboratories, Abbott Park, IL and Abbott, GmbH Diagnostika, Weisbaden, Germany). Anti-HCV IgM was determined by utilizing a solid phase enzyme immunoassay ("IgM Combo", available from Abbott, GmbH Diagnostika, Weisbaden, Germany). The anti-HCV IgM assay utilized recombinant antigens derived from the structural (core) and non-structural (NS3/NS4) part of the virus. These methods are detailed in Chau et al., J. Virol. Methods. 35:343-352, 1991). The HCV antigens included clone c100-3 (as described by Kuo et al., Science 244:362-364 [1989]) chimeric polypeptide expressed in yeast, plus recombinant HCV polypeptides expressed in E. coli that included those from pHCV-23 (c100 fragment, lacking the first 107 N-terminal amino acids), pHCV-29 (CKS-33c), pHCV-34 (CKS-CORE) and pHCV-35 (λ pL CORE), and pHCV-45 (NS4/NS5 junction). The amino acids sequences of these HCV polypeptides are known in the art and are described in European Patent Application 0 388 232, published September 19, 1990 (see pages 32 and 34).

All HCV proteins were expressed as CMP-KDO synthetase (CKS) fusion proteins (as taught by T. J. Bolling and W. Mandecki, "An Escherichia coli expression vector for high-level production of heterologous proteins in fusion with CMP-KDO synthetase," Biotechniques 8:488-490 [1990]), with the exception of pHCV-35, which was expressed in a lambda (λ) pL expression system.

Anti-HCV IgM results were expressed as specimen absorbance divided by mean absorbance (both absorbance measured at 492 nm) of three (3) negative controls (S/N). In 450 healthy blood donors, the mean S/N value was 1.0 ± 0.5 (mean S/N ± 1 standard deviation [SD]). Samples with S/N ≥ 4 were characterized further for their IgM antigen

specificity using single bead HCV IgM assays utilizing the HCV-Core, HCV-NS3 and HCV-NS4 antigens.

A neutralization assay using a different recombinant construct was used for confirmation testing. Rheumatoid factor interference was eliminated by modifying the specimen diluent as taught hereinabove, which allowed the separation of IgG antibodies.

3. Results

Before IFN therapy anti-HCV IgM values were determined to be significantly higher in NR than in CR (t-test). In contrast, no significant difference was seen for serum ALT levels in NR and CR before IFN therapy (t-test). In patients with complete response to IFN therapy, decreasses of HCV IgM and ALT levels before, during and after therapy was observed. In patients who exibited no response to IFN therapy, HCV IgM and ALT levels both remained elevated. Nineteen of twenty-three (83%) patients of the NR group had high or increasing HCV IgM levels throughout IFN therapy. It also was observed that in both the NR and CR anti-HCV IgM response was directed almost always against the structural (Core) part of the virus. Low level anti-C100 or anti-33c IgM was found only in 1/34 patients tested. These data are summarized in the following Table 1 which summarizes HCV IgM values (S/N) and ALT levels seen in chronic hepatitis C Patients with no response, and in Table 2 which summarizes HCV IgM values (S/N) and ALT levels seen in chronic hepatitis C Patients with complete response

Table 1
(No Response To IFN, No. Patients = 23 )

| | Before IFN Therapy | At start of IFN Therapy | At end of IFN Therapy | After IFN Therapy |
|---|---|---|---|---|
| Mean IgM (S/N) ±SD | 13.9 ± 13.8 | 13.5 ± 13.7 | 13.8 ± 13.8 | 16.8 ± 13.8 |
| No. Samples | 23 | 22 | 22 | 13 |
| Mean ALT (IU/ML) ±SD | 88 ± 35 | 66 ± 25 | 81 ± 58 | 86 ± 42 |
| No. Samples | 16 | 22 | 22 | 20 |

Table 2
(Complete Response to IFN, No. Patients = 11)

| | Before IFN Therapy | At start of IFN Therapy | At end of IFN Therapy | After IFN Therapy |
|---|---|---|---|---|
| Mean IgM (S/N) ±SD | 6.3 ± 6.0 | 4.4 ± 3.4 | 3.6 ± 4.3 | 2.2 ± 1.4 |
| No. Samples | 10 | 11 | 11 | 7 |
| Mean ALT (IU/ML) ±SD | 130 ± 163 | 18 ± 13 | 15 ± 14 | 15 ± 10 |
| No. Samples | 8 | 11 | 10 | 8 |

4. Conclusions

From the data presented herein, anti-HCV IgM (core) levels can predict response to IF therapy. It also was observed that decreasing anti-HCv IgM (core) levels can be an early indicator for relapse during and after IFN therapy. Also, it was observed that increasing anti-HCv IgM (core) levels can be an early indicator for relapse during and after IFN therapy. Finally, anti-HCv IgM can give additional diagnostic information in conjuction with ALT serum levels for patients with chronic hepatitis C infections.

## Example 2
## Anti-GOR IgG Activity

1. Patient Selection and Categorization

Thirty-three (33) patients with chronic active hepatitis C at different time points during interferon therapy (1 to 5 million units of interferon alpha-2B, three times a week, for at least six (6) months) were included. Complete responders (CR) (11/33) were defined by normalization of serum transaminases during treatment. Non-responders (NR) (22/33) were defined as those individuals who showed transaminases levels above two (2) times the normal values (28 IU/ML). Samples were tested for GOR IgG and GOR IgM antibodies. Samples were collected approximately one (1) month before, two (2) months after the beginning of and at the completion of IFN therapy. In a number of patients, samples drawn approximately six (6) months after therapy also were available.

2. GOR Detection in Samples

All samples initially were tested by the Abbott HCV 2nd Generation assay EIA and the Abbott HCV Supplemental EIA (available from Abbott Laboratories, Abbott Park, IL and Abbott, GmbH Diagnostika, Weisbaden, Germany). Anti-HCV IgM was determined by utilizing a solid phase enzyme immunoassay ("IgM Combo", available from Abbott, GmbH Diagnostika, Weisbaden, Germany). The anti-HCV IgM assay utilized recombinant antigens derived from the structural (core) and non-structural (NS3/NS4) part of the virus. These methods are detailed in Chau et al., J. Virol. Methods. 35:343-352, 1991). The HCV antigens included clone c100-3 (as described by Kuo et al., Science 244:362-364 [1989]) chimeric polypeptide expressed in yeast, plus recombinant HCV polypeptides expressed in E. coli that included those from pHCV-23 (c100 fragment, lacking the first 107 N-terminal amino acids), pHCV-29 (CKS-33c), pHCV-34 (CKS-CORE) and pHCV-35 (λ pL CORE), and pHCV-45 (NS4/NS5 junction). The amino acids sequences of these HCV polypeptides are known in the art and are described in European Patent Application 0 388 232, published September 19, 1990 (see pages 32 and 34).

All HCV proteins were expressed as CMP-KDO synthetase (CKS) fusion proteins (as taught by T. J. Bolling and W. Mandecki, "An Escherichia coli expression vector for high-level production of heterologous proteins in fusion with CMP-KDO synthetase," Biotechniques 8:488-490 [1990]), with the exception of pHCV-35, which was expressed in a lambda (λ) pL expression system.

Anti-GOR IgG and IgM levels were determined by the assay method for IgG described hereinabove and disclosed in S. U. Mehta et al., J. Clin. Immunol. 12 (3):178-183 (1992). When assaying for anti-GOR IgM levels, the appropriate rare reagents were those as described for the HCV IGM assay, but utilizing the synthetic peptides spGOR346 and spGOR2.

3. Results

It was found that 11 patients had normalized ALT levels at the end of therapy (CR). Non-responders (NR) numbered 22. It was observed that eight of eleven (73%) of CR showed decrease of anti-GOR IgG. Further, anti-GOR IgM was higher in NR than CR before

therapy, while there were no significant changes in this value during therapy.

It thus is possible to monitor the effectivness of interferon therapy during its use in individuals infected with HCV, especially those who have been diagnosed with chronic acute HCV infection. The amount of anti-HCV IgM pre-interferon therapy and/or anti-GOR IgG pre-interferon therapy is compared to the amount of of anti-HCV IgM pre-interferon therapy and/or anti-GOR IgG pre-interferon therapy during therapy. A decrease in the level of HCV IgM antibodies or anti-GOR IgG antibodies during therapy is an indication that the patient is responding to the interferon therapy. Also, it is possible that dosages may be varied depending upon the results obtained by this monitoring. The embodiments described and presented herein are intended as examples rather than as limitations. Thus, the description of the invention is not intended to limit the invention to the particular embodiments disclosed, but it is intended to encompass all equivalents and subject matter within the spirit and scope of the invention as described and contemplated above, and as set forth in the following claims.

WHAT IS CLAIMED IS:

1. An method for determining the effectiveness of interferon therapy in an individual infected with HCV, comprising:

(a) determining the pre-interferon amount of anti-HCV IgM or anti-GOR IgG in a test sample of a patient infected with HCV;

(b) determining the amount of anti-HCV IgM and/or anti-GOR IgG in an individual during inteferon therapy by assaying for anti-HCV IgM or anti-GOR IgG in at least one other test sample;

(c) comparing the pre-interferon amount of anti-HCV IgM or anti-GOR IgG with the amount of anti-HCV IgM or anti-GOR IgG present in a different test sample obtained from the individual during interferon therapy;

wherein a decrease in anti-GOR IgG and/or anti-HCV IgM indicates complete response in said individual.

2. The method of claim 1, wherein the GOR antigen is selected from the group consisting of GOR2 and GOR346.

3. The method of claim 1, wherein the HCV antigen is selected from the group consisting of HCV-core, HCV-33c and HCV-c-100.

4. The method of claim 1 wherein said HCV antigen is HCV-core.

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07287

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) :G01N 33/576; C12Q 1/70; A61K 37/66
US CL :435/5, 7.1; 436/513, 536; 424/85.4, 85.5, 85.6, 85.7
According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/5, 7.1; 436/513, 536; 424/85.4, 85.5, 85.6, 85.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE, APS

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | Journal of Clinical Immunology, Volume 12, No. 3, issued 1992, S. U. Mehta et al, "Immune Response to GOR, a Marker for Non-A, Non-B Hepatitis and Its Correlation with Hepatitis C Virus Infection", pages 178-184, see page 178. | 1,2 |
| Y | Journal of Clinical Microbiology, Volume 29, No. 3, issued March 1991, G. J. Dawson et al, "Detection of Antibodies to Hepatitis C Virus in U.S. Blood Donors", pages 551-556, see pages 551, 552 and 554. | 1-4 |

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

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| * | Special categories of cited documents: | "T" | later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention |
| "A" | document defining the general state of the art which is not considered to be part of particular relevance | "X" | document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone |
| "E" | earlier document published on or after the international filing date | "Y" | document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art |
| "L" | document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) | "&" | document member of the same patent family |
| "O" | document referring to an oral disclosure, use, exhibition or other means | | |
| "P" | document published prior to the international filing date but later than the priority date claimed | | |

| | |
|---|---|
| Date of the actual completion of the international search<br>20 OCTOBER 1993 | Date of mailing of the international search report<br>26 OCT 1993 |
| Name and mailing address of the ISA/US<br>Commissioner of Patents and Trademarks<br>Box PCT<br>Washington, D.C. 20231<br>Facsimile No. NOT APPLICABLE | Authorized officer<br>KAY K. KIM, PH.D.<br>Telephone No. (703) 308-0196 |

Form PCT/ISA/210 (second sheet)(July 1992)*

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/07287

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | Biochemical and Biophysical Research Communications, Volume 172, No. 2, issued 30 October 1990, K. Muraiso et al, "A STRUCTURAL PROTEIN OF HEPATITIS C VIRUS EXPRESSED IN E. Coli FACILITATES ACCURATE DETECTION OF HEPATITIS C VIRUS" pages 511-516, see page 511 and 514. | 1-4 |
| Y | Journal of Clinical Microbiology, Volume 27, No. 5, issued May 1989, D. W. T. Ho et al, "Rapid Diagnosis of Acute Epstein-Barr Virus Infection by an Indirect Enzyme-Linked Immunosorbent Assay for Specific Immunoglobulin M (IgM) Antibody without Rheumatoid Factor and Specific IgG Interference", pages 952-958, see page 952. | 1,3,4 |
| A | Proc. Natl. Acad. Sci., USA, Volume 82, issued April 1985, Y. K. Shimizu et al, "Production of antibody associated with non-A, non-B hepatitis in a chimpanzee lymphoblastoid cell line established by in vitro transformation with Epstein-Barr virus", pages 2138-2142. | 1-4 |
| A,P | US, A, 5,191,064 (Arima et al) 02 March 1993. | 1-4 |